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Introduction

Novel methods for integrated risk assessment of cumulative stressors — Results from the NoMiracle project

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ABSTRACT

This special issue covers the main results of the European Sixth Framework Integrated Research project NoMiracle (Novel Methods for Risk Assessment of Cumulative stressors in Europe). New tools to analyse, characterise and quantify the combined risks to health or the environment from multiple stressors are presented or reviewed. Examples of cumulative stressors are mixtures of chemicals alone or in combination with biological or physical environmental factors such as pathogens and climate extremes. Among the main findings, the scientific work points at the importance of time in dealing with toxicity, and in particular the toxicity of chemical mixtures, the natures of the uncertainties associated with risk assessment and the value of visualisation in identifying and quantifying the most relevant risks. A major conclusion of the project is that researchers and regulators should focus on the receptor rather than on the single stressor or combination of agents. There is also a need for more efforts on mechanistic understanding and for a mechanism-based framework for interpreting mixture/multiple stressor effects.
The new tools are available on the internet (http://nomiracle.jrc.ec.europa.eu).

1. Introduction

Living organisms are never subject to single stressors at set doses, but rather to a complex array of physical, chemical and biological environmental stressors. This issue deals with the project NoMiracle: Novel Methods for Integrated Risk Assessment of CumuLative stressors in Europe. The project addresses the problems of assessing combined risks to health or the environment from multiple stressors (NoMiracle, 2010). From 2004 to 2009 a team involving contributions from more than 100 scientists and 28 PhD students from 38 institutions in 17 European countries have worked together to develop new methods for assessing the cumulative risks from combined exposures to several stressors including mixtures of chemical and physical/biological agents. The work has been granted under the European 6th Framework Programme Priority [1.1.6.3] 'Global Change and Ecosystems', Topic VII.1.1.a 'Development of risk assessment methodologies'.

In this issue key results and lessons learned are compiled and set in perspective in review articles, with selected new approaches also illustrated in more specific papers. The contributions are divided into three parts dealing with Effects of combined stressors, Fate and exposure of chemicals and mixtures, and Risk assessment and risk governance, respectively. Key tools arising from the project have been compiled into a 'NoMiracle Tool Box', a short description of which is presented in this introduction.

2. Effects of combined stressors

One of the main tasks of NoMiracle has been to develop a research framework for the description and interpretation of cumulative exposure and effect. This conceptual effort is described in this issue by Spurgeon et al. under the title "Systems toxicology approaches for understanding the joint effects of environmental chemical mixtures — model and non-model species". They suggest a three stage schema (Table 1), which allows for a more easy design of experimental approaches:

Building on this conceptual framework, the effect assessment of mixtures of chemicals is still an almost infinite and unrealistic task. Assuming that around 70,000 chemicals are in regular use worldwide, a 'full' assessment of theoretical binary mixtures would require 2.45×10^{9}, and of all ternary combinations 5.7×10^{13} test packages. During the work in NoMiracle, it gradually became clear that the current approach in coping with chemical mixtures focussing on "the chemical" and "chemical cocktails" should be replaced by a focus on the biological receptor, e.g. on the organism (man or other species), the population or the ecosystem being exposed to a more definite cocktail of stressors.

At the outset of the project it was predefined within the initial scope that focus should be on chemicals with specific mode of action, not including hormone mimicking or carcinogenic compounds which already have undergone a significant research. Such specifically acting

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Table 1
Research framework for the description and interpretation of cumulative exposure and effect.

Three stage scheme
1. Interactions in the environmental media that may modulate the environmental availability of one or more chemicals (environmental availability).
2. Interactions at site of uptake and/or elimination of the chemical from the organisms that may result in the modulation of the total accumulated internal concentration/dose or interactions that change detoxification/ bioactivation and/or compartmentalization of one or more chemicals (toxicokinetics including uptake and elimination and biotransformation).
3. Interactions at the target site that may affect the binding of one of more chemicals to a receptor through which toxicity may be (partly) mediated (toxicodynamics).

chemicals, as well as more widely acting and narcotic chemical groups may interact with other classes of compounds, by for example changing uptake and excretion processes, or by inhibiting or activating one or more enzyme families. Additional to these exposures, the receptor meets natural stressors and often relatively high concentrations of non-specific acting chemicals as illustrated in Fig. 1.

In the NoMiracle project, a series of existing testing guidelines with species ranging from one-celled organisms to mammals, have been modified for testing chemical mixtures of a variety of compounds counting pesticides, biocides, pharmaceuticals, and metals. To date, the work has resulted in 272 different exposure/ endpoint measurement datasets. When analysed against the different models for mixture effect prediction available, such as concentration addition and independent action, a total of 292 different data analyses have been conducted on the basis of this data. Of these initial analyses, 213 of the mixture studies assessed have been found to have statistically or biologically significant interactions. This number of interactions, while seemingly high, is actually in rather good agreement with previous studies that have investigated the utility of the classical concentration addition (CA) and the independent action (IA) models to describe binary effects (Cedergreen et al., 2008). Analysis to identify the potential causes of observed toxicity and where present interactions between chemicals has highlighted dysfunction at all levels of biological organisation and the causes of the effects seen. Response patterns have been compiled between

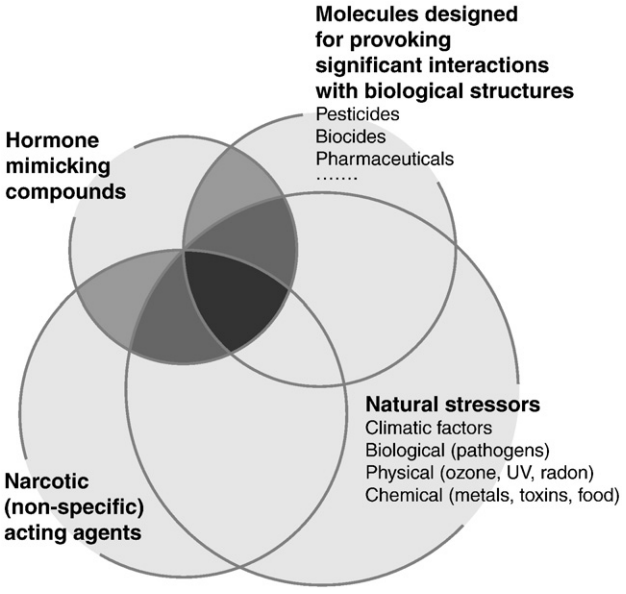

Fig. 1. Illustration of possible interactions of multiple stressors.

human cell lines and ecological species, and by developing new approaches to the interpretation of immuno-toxicological data sets.

Another major understanding reached during the research was the importance of time in dealing with toxicity, and in particular the pattern of toxicity for chemical mixtures. Baas et al. (this issue) explains in the article "Understanding toxicity as processes in time" how ignorance of the time factor can lead to severe bias in environmental risk assessment, and they suggest how current test guidelines may be adapted to yield data on time dependency. To this end, there is a need for process based models capable of extrapolation with use of limited data. In the NoMiracle project such a new model for mixture toxicity assessment has been developed, based on the Dynamic Energy Budget theory (DEB) concept. In this issue, Baas et al. review DEB in assessing toxic effects of mixtures. The DEB model provides a single consistent framework for the interpretation of mixture effects and a systematic approach for multiple chemical effect prediction based on minimal data, and by integrating all endpoints and combining toxicokinetics and toxicodynamics. This model further allows for prediction of mixture effects based on a reduced amount of data, but calls for more experimental effort on elucidating mechanistic processes and providing time-dependent toxicity data rather than current toxicological testing using one time-point.

An important part of the NoMiracle project was to include the development of protocols for testing the interaction of chemical and natural stressors, since these represent important ecological scenarios that are largely neglected in standardised testing. In this issue Holmstrup et al. review the area and conclude that synergistic or antagonistic interactions are frequent between the natural stressors and the chemicals tested. The review counts more than 150 studies of which 25 were conducted in the NoMiracle project, thus contributing considerable to this field. The ecotoxicological studies included heat stress, freezing, desiccation, oxygen depletion, starvation and pathogens. In particular, the climate related stressors are important for risk assessment of the impact of climate change on ecosystems. The review of the influence of inflammatory stress factors on the immune-modulatory effects of chemicals in human cell lines showed that about 70% of the tested chemicals may potentially compromise the human immune system. The knowledge from these studies should be generalised and subjected to a detailed uncertainty analysis as a step towards improving the scientific basis of the current safety factors utilised in risk assessment. Based on data produced in NoMiracle in combination with older data, Laskowski et al. made a metaanalysis and case studies on the interactions between toxic chemicals and natural environmental factors (this issue). They found very high evidence for interactions and argue for the inclusion of natural stressors in the design of second-tier ecological risk assessment.

The work in NoMiracle included comprehensive mechanistic analyses by use of global gene, protein and metabolite analysis technologies on a series of animal phyla, including human cell lines. The present issue gives an example of the use of transcriptomic and proteomic effects of a neonicotinoid insecticide mixture in a marine mussel (Dondero et al., this issue). This detailed mechanistic study reveals that beyond the same mode of action of single chemicals, mixtures may show different and species-dependent toxicodynamics, and thus affect the toxic outcome of the mixture. This key theme of the role of toxicokinetic in mixture toxicity, in this case driven by molecular based analysis of the genes and proteins involved, highlights the potential of the relevant biochemical systems and pathways as points at which multiple chemicals can interact within the body. The use of high-throughput molecular approaches such as transcriptomics, proteomics and metabolomics may contribute to mechanistic understanding of the toxicodynamics of mixtures and allow for better prediction of mixture toxicity.

Methods for studying uptake and elimination kinetics have been further developed in the NoMiracle project, allowing for prediction of biodegration pathways for organic chemicals including the formation